

Cancer Nano (2010) 1:29–34  
DOI 10.1007/s12645-010-0004-2

## ORIGINAL PAPER

# Evaluating the potential of polymer nanoparticles for oral delivery of paclitaxel in drug-resistant cancer

V. Bhardwaj · J. A. Plumb · J. Cassidy ·  
M. N. V. Ravi Kumar

Received: 24 June 2010 / Accepted: 1 August 2010 / Published online: 7 September 2010  
© Springer-Verlag 2010

**Abstract** The present study was designed to explore the ability of polymeric nanoparticles to restore drug sensitivity to P-glycoprotein over-expressing cancer cells. A multidrug-resistant cell line 2780 AD and its sensitive parent cell line A2780 were studied in cell culture and as a xenografted tumour model. Paclitaxel was incorporated in poly(lactide-*co*-glycolide) nanoparticles of average diameter 125 nm stabilised by a positively charged surfactant. The nanoparticulate formulation was shown to be about sevenfold more potent than free paclitaxel against cell line A2780 and the poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles alone were nontoxic to the cells at the concentrations required to deliver the drug. Whilst the oral formulation of paclitaxel was not as potent as the free drug in the A2780 xenografts, it showed significant activity against 2780 AD tumours, which are resistant to the maximum tolerated intravenous dose of paclitaxel. The efficacy of orally delivered paclitaxel in this drug-resistant model supports the concept of exploring nanoparticles for improved drug delivery.

**Keywords** P-glycoprotein · EPR effect · Nanoparticles · Oral drug delivery · Paclitaxel

## 1 Introduction

Drug resistance is a common cause of treatment failure in anticancer therapy. One mechanism of drug resistance identified in studies with cell lines is through expression of drug transporters that pump the drug out of the cells. Paclitaxel is one of the most effective drugs against cancer. However, its clinical utility is limited due to unfavourable solubility in water and poor permeability across the biological barriers. Furthermore, it is extensively pumped out by the P-glycoprotein (Pgp; Roy and Horwitz 1985). Due to these reasons, the drug shows poor bioavailability when administered orally. Therefore, it is typically administered intravenously in a mixture of cremophor EL™ and alcohol, but this vehicle can cause anaphylactoid reactions (Dye and Watkins 1980).

Novel drug delivery strategies are being explored for delivery of paclitaxel. Small particles can improve the oral bioavailability of medicinal compounds (Florence 1997; Sakuma et al. 2001). The particles are believed to be delivered to the systemic circulation through the lymphatic system (Sakuma et al. 2001; Eldridge et al. 1990). Due to their profoundly different pharmacokinetics and cellular disposition, they may be considered as implanted mobile depots of drugs and offer many unexplored benefits.

A2780 is a human ovarian carcinoma cell line that is sensitive to doxorubicin and many other cytotoxic drugs including paclitaxel. 2780 AD is a multidrug-resistant variant of A2780 that was developed by repeated exposure to

---

V. Bhardwaj · M. N. V. Ravi Kumar (✉)  
Strathclyde Institute of Pharmacy and Biomedical Sciences,  
The John Arbuthnott Building, University of Strathclyde,  
27 Taylor Street,  
Glasgow G4 0NR, UK  
e-mail: mnvrkumar@strath.ac.uk

J. A. Plumb (✉) · J. Cassidy  
The Centre for Oncology and Applied Pharmacology,  
Cancer Research UK, Department of Medical Oncology,  
University of Glasgow,  
Garscube Estate, Switchback Road,  
Glasgow G61 1BD, UK  
e-mail: j.plumb@beatson.gla.ac.uk

doxorubicin. The main mechanism of resistance is over-expression of the Pgp, and this resistance is shared towards other anticancer drugs that are substrates (Dantzig et al. 1996). 2780 AD is about 2,000-fold resistant to paclitaxel compared to the parental A2780 cell line. When grown as xenografts in athymic nude mice, A2780 shows a significant growth delay when the mice are treated with paclitaxel. By contrast, tumours of 2780 AD are resistant to the maximum tolerated dose of paclitaxel. However, 2780 AD can be sensitised to paclitaxel by treatment of the mice with an inhibitor of the drug efflux pump (Mistry et al. 2001).

Liposomes have been shown to increase the accumulation of doxorubicin in MDR Chinese hamster LZ, human breast cancer MCF-7/ADR, and the ovarian carcinoma SKVLB cell line as a result of changes in the intracellular vesicular transport (Thierry et al. 1993). In a study with drug-resistant cell lines, it has been shown that doxorubicin encapsulated in polymer–lipid nanoparticles was accumulated within the cell to a higher degree compared to free doxorubicin (Wong et al. 2006).

Lipid partitioning of the substrate in the membrane structure has been postulated as essential for efflux, and the lipid type can affect the interaction with the substrate (Romsicki and Sharom 1999). Liposomes increased vinblastine accumulation in resistant human lymphoblastic leukemic cell CEM/VLB<sub>100</sub> even when the drug was co-administered in free form rather than incorporated within the liposomes, probably due to amalgamation of lipidic components into cell membrane lipids and subsequent alteration in membrane fluidity (Warren et al. 1992). Pluronic block copolymers have been shown to sensitise MDR cell lines to cytotoxics (Kabanov et al. 2002). A micellar formulation of pluronic doxorubicin conjugate SP1049C is presently undergoing Phase II clinical trials for Pgp-targeted therapy (Valle et al. 2010).

Polymeric nanoparticles have been documented to reverse drug resistance in cancer cell lines based on the hypothesis of transport through endocytic pathways (Cuvier et al. 1992). However, local high concentrations of drug released from polymeric nanoparticles adsorbed on to resistant P388/ADR cell surface have also been proposed to reverse resistance (de Verdière et al. 1997). A coculture of these cells with macrophages showed beneficial effects especially when combined with cyclosporin A, which is an inhibitor of the Pgp efflux transporters (Soma et al. 1999). The cytotoxic and the inhibitor have been encapsulated into a single nanoparticulate carrier system (Emilienne Soma et al. 2000). Curiously, it was believed that nanoparticles of 185 nm cannot be of utility in solid tumours implanted subcutaneously in animals, because they would not diffuse out of the vascular endothelium and migrate to the cancer cells (de Verdière et al. 1997).

We have reported development of polymeric nanoparticles incorporating paclitaxel which showed activity in a chemically induced breast tumour model in rats when administered orally (Bhardwaj et al. 2009). In this study, we have examined the ability of these orally delivered nanoparticles to inhibit the growth of human ovarian tumours grown subcutaneously in athymic nude mice.

## 2 Material and methods

### 2.1 Material

Paclitaxel was obtained from Samyang Genex (as Genexol®) (Seoul, South Korea) and Sigma-Aldrich (St. Louis, MO, USA). Didodecyldimethylammonium bromide (DMAB) was purchased from Fluka Buchs SG, Switzerland). Poly(lactic-co-glycolic acid) (PLGA) 50:50 block copolymer (RG 503H, molecular weight 35–40 kDa) was procured from Boehringer Ingelheim KG (Ingelheim, Germany). All other chemicals were purchased from Sigma-Aldrich and were used as obtained.

### 2.2 Preparation of paclitaxel-loaded nanoparticles

Drug-loaded nanoparticles were prepared with initial drug loading of 5% (w/w of PLGA) paclitaxel. In brief, 2.5 mg paclitaxel was dissolved in 5 ml ethyl acetate and 50 mg PLGA was added to this solution and stirred for 2 h. This solution was poured with stirring in 5 ml of aqueous 1.0% w/v DMAB solution. The primary emulsion so obtained was homogenised for 5 min by a shaft-type tissue homogeniser at 15,000 rpm (Polytron 4000, Kinematica, Switzerland). Finally, this emulsion was diluted six times with water and stirred at 800 rpm with a magnetic bar for 4–6 h to remove the ethyl acetate. Nanoparticle suspension obtained was washed twice by centrifugation to remove the unbound drug and surfactant. The prepared formulation was characterised for size, zeta potential and polydispersity index (PDI) using Zetasizer (Nano-ZS, Malvern, UK). Analysis of paclitaxel was carried out by high-performance liquid chromatography (HPLC, Waters, USA) using ultraviolet detection at 227 nm on a 250×4.6 mm reverse phase C18 (Symmetry or Lichrocart) column. A mobile phase consisting of methanol, acetonitrile and 5 mM phosphate buffer (pH 2.5) in the ratio 70.0:2.5:27.5 eluted paclitaxel from a 20 µl injection in 7–8 min when pumped at 0.7 ml/min.

### 2.3 Cell culture

The human ovarian carcinoma cells, A2780, and its drug-resistant variant, 2780 AD, were provided by Dr. T. C. Hamilton (Fox Chase Center, Philadelphia, PA). The

2780 AD cells were resistant to both doxorubicin and paclitaxel (Mistry et al. 2001). They were maintained in Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing glutamine (2 mM) and foetal calf serum (10%).

Cells were grown to ~90% confluence in 75 cm<sup>2</sup> T-flasks. Culture medium was changed on alternate days, and cells were cultured at a temperature of ~37°C in an atmosphere of ~85% relative humidity and ~5% CO<sub>2</sub>.

#### 2.4 Cytotoxicity assay

Drug sensitivity was determined by a tetrazolium dye-based assay (MTT; Plumb et al. 1989). Cells were seeded at a density of  $2 \times 10^3$  (2780 AD) or  $1 \times 10^3$  (A2780) cells/well in 96-well flat-bottomed plates (Iwaki from Bibby, Bicester, Oxon) and allowed to attach and grow for 48 h. The cells were exposed to drug for 24–72 h. Nanoparticles containing paclitaxel were either prepared under sterile conditions or filtered through a 0.22- $\mu$ m filter. Paclitaxel in dimethyl sulphoxide (DMSO), polymeric nanoparticles, and polymeric nanoparticles containing paclitaxel were diluted in cell culture medium for use. After a defined incubation time, drug was removed and the cells grown in fresh medium for 72 h, after which cells were fed with medium and MTT (50  $\mu$ l, 5 mg/ml) was added to each well. Plates were incubated in the dark at 37°C for 4 h, medium was removed, and MTT formazan crystals were dissolved in DMSO (200  $\mu$ l/well). Glycine buffer (25  $\mu$ l/well, 0.1 M, pH 10.5) was added to make the contents alkaline, and the absorbance was measured at 570 nm in a multiwell plate reader (Emax, Molecular Devices with Softmax Pro analytical software). A typical dose–response curve consisted of eight drug concentrations, and four wells were used per drug concentration. Sensitivity is expressed as the IC<sub>50</sub> (mean  $\pm$  SEM of three experiments) defined as the concentration of drug required to reduce the absorbance of the wells to 50% of that of the control untreated cells.

#### 2.5 Efficacy studies in tumours in vivo

Animal studies were carried out under an appropriate United Kingdom Home Office Project Licence, and all work conformed to the UKCCR Guidelines for the welfare of animals in experimental neoplasia. Monolayer cultures were harvested with trypsin/ethylenediaminetetraacetic acid (EDTA) and resuspended in phosphate buffered saline (PBS). About  $10^7$  cells were injected subcutaneously into the right flank of athymic nude mice (CD1 *nu/nu* mice from Charles River). After 7 to 10 days when the mean tumour diameter was at  $\geq 0.5$  cm, animals were randomised in groups of 6 for experiments (Workman 2010). Mice received either no treatment, paclitaxel (15 mg/kg body

weight, i.v.) in cremophor EL and ethanol mixture (5%:5%) diluted in dextrose solution (90%), blank nanoparticles orally (dose equivalent of drug-loaded) or the drug-loaded nanoparticulate formulation orally containing paclitaxel equivalent to 15 mg/kg body weight. Mice were weighed daily, and tumour volumes were estimated by caliper measurements assuming spherical geometry ( $\text{volume} = d^3 \times \pi/6$ ).

### 3 Results

#### 3.1 Preparation of paclitaxel-loaded nanoparticles

Particles prepared with 1% DMAB with an initial load of 5% w/w of paclitaxel relative to the polymer weight had an average particle size of about 120 nm (Bhardwaj et al. 2009). Particles were positively charged as indicated by zeta potential values of 50–60 mV. The particle washing did not appreciably increase the particle size (mean average particle size 130 nm).

#### 3.2 Cytotoxicity of the nanoparticles

The cytotoxicity of paclitaxel and the drug-loaded nanoparticles is shown in Table 1. A2780 is about 2,000-fold more sensitive to paclitaxel than the drug-resistant derivative (IC<sub>50</sub>  $2.26 \pm 0.91$  nM for A2780 cf.  $5240 \pm 637$  nM for 2780 AD). The blank nanoparticles were relatively nontoxic to the cells with an IC<sub>50</sub> based on the theoretical paclitaxel load of  $1774 \pm 129$  nM. Formulation of paclitaxel with the nanoparticles increased the toxicity of the drug to the drug-sensitive cell line A2780 by about sevenfold (free drug  $2.26 \pm 0.91$  cf. nanoparticle  $0.30 \pm 0.03$  nM). The paclitaxel sensitivity of the drug-resistant derivative was increased by prolonged exposure to the drug (Table 1). However, the nanoparticles themselves were toxic to the cells at the

**Table 1** Paclitaxel sensitivity of cell lines A2780 and the drug-resistant derivative 2780AD to paclitaxel and paclitaxel nanoparticles

	IC <sub>50</sub> (nM)		
	A2780	2780 AD	
	24 h	24 h	72 h
Blank nanoparticles	$1,774 \pm 129$	–	$3,175 \pm 79$
Paclitaxel	$2.26 \pm 0.91$	$5,240 \pm 637$	$2,013 \pm 73$
Paclitaxel nanoparticles	$0.30 \pm 0.03$	$1,661 \pm 89$	$903 \pm 49$

Sensitivity is expressed as the IC<sub>50</sub> (mean  $\pm$  SEM of three estimations) defined as the concentration of drug required to reduce the absorbance of the wells to 50% of that of the control untreated cells

concentrations required to achieve paclitaxel  $IC_{50}$  concentration ( $3175 \pm 79$  nM for the blank particles of the  $IC_{50}$  for paclitaxel of  $2013 \pm 73$  nM).

### 3.3 Efficacy of the nanoparticles in human tumour xenograft models

The effect of paclitaxel and the drug-loaded nanoparticles on the growth of tumours derived from the drug-sensitive A2780 cell line is shown in Fig. 1. Paclitaxel treatment produces a significant growth delay with no apparent toxicity as measured by changes in body weight. The drug-loaded nanoparticles when given orally also inhibit the growth of the tumours, but the growth delay is not as marked as with the intravenous preparation. Tumours derived from the drug-resistant variant of A2780 (2780 AD) are resistant to the maximum tolerated dose of the intravenous paclitaxel preparation ( $15 \text{ mg/kg} \times 3$ ). However, in this model, treatment with the paclitaxel-loaded nanoparticles given orally results in a growth delay comparable to that seen in the drug-sensitive

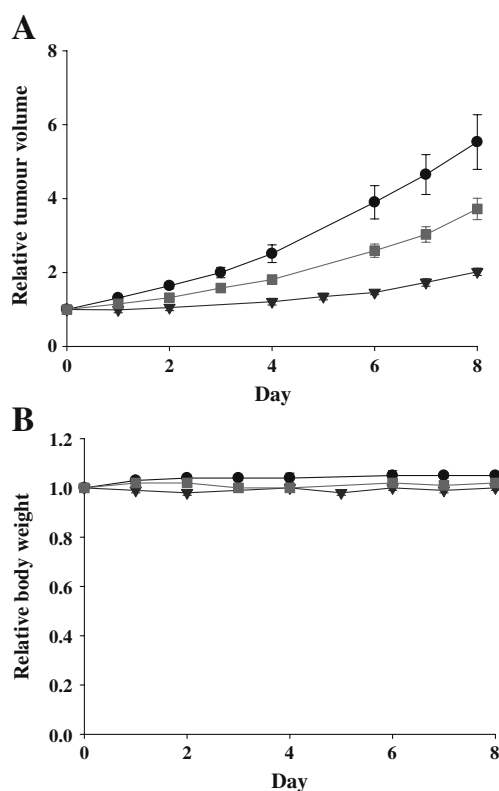
A2780 model with this formulation. The blank nanoparticles alone have no effect on tumour growth and are nontoxic to the mice as measured by effects on the body weight and behaviour.

## 4 Discussion

We have shown clearly that the paclitaxel-loaded nanoparticles are able to deliver paclitaxel to the tumour when given by the oral route. Furthermore, the nanoparticles can partially circumvent multidrug resistance in vivo. PLGA is a biodegradable and biocompatible polymer approved by the US Food & Drug Administration for human use and is a popular material for preparing nanoparticles for drug delivery. A nanoparticulate formulation has been shown to enable oral delivery of otherwise poorly bioavailable paclitaxel and impart improved efficacy (Bhardwaj et al. 2009). The present study supports and extends these observations.

The paclitaxel-loaded nanoparticles retained activity in cell lines in vitro. Interestingly, the formulation was able to increase the sensitivity of the already relatively sensitive cell line A2780 by about sevenfold (Table 1). The nanoparticles were nontoxic to the cells at the concentrations required to deliver sufficient paclitaxel to the cells. For comparison, the  $IC_{50}$  concentrations of the blank nanoparticles is expressed in paclitaxel equivalents, i.e. the amount needed to give the required concentration of paclitaxel. Since the  $IC_{50}$  of the blank particles is about 500-fold greater than the concentration required to deliver the  $IC_{50}$  concentration of paclitaxel (Table 1), the increased toxicity of the drug-loaded nanoparticles cannot be explained by toxicity of the nanoparticle itself. By contrast, the drug-resistant variant 2780 AD is about 2,000-fold resistant to paclitaxel, and in order to deliver this concentration of paclitaxel, the nanoparticles are used at close to their toxic concentration. Thus we cannot exclude the possibility that the increased activity in this cell line is due to combined toxicity of the paclitaxel and the nanoparticles.

The observed  $IC_{50}$  values of the blank nanoparticles in the drug-resistant cell line raises questions about the inherent cytotoxicity of the polymeric nanoparticles per se. One possible explanation is that the particles, merely with their physical presence beyond a particular threshold, are interfering with structural integrity dynamics or metabolic process of the cells. An alternate hypothesis could be that they are making a physical barrier on the surface of cells that inhibits transport of nutrition across the cells, and this effect is dependent on the concentration. Clearly, the washing of the well contents by aspiration of medium is ineffective in disturbing this association with the cells. The particles being positively charged are expected to interact



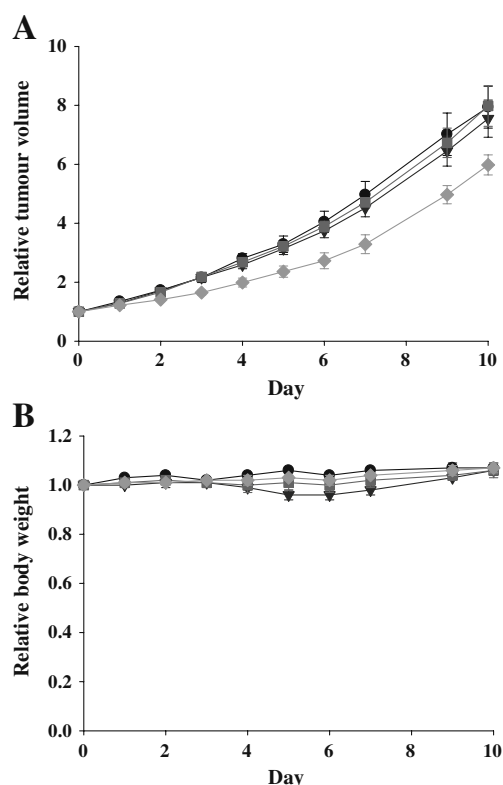
**Fig. 1** The effect of paclitaxel and paclitaxel nanoparticles on the growth of tumours of cell line A2780 when grown as xenografts in mice. Mice were treated once tumours reached a mean diameter of 0.5 cm. Groups of six mice were either untreated (●), given paclitaxel (dissolved in cremophor EL, ethanol, dextrose) intravenously (▼), or paclitaxel-loaded nanoparticles orally (■) on days 0, 2, and 4. Tumour volumes (A) and body weights (B) are expressed relative to that on the day of treatment (day 0) and results are the mean  $\pm$  SEM of six mice

with the negatively charged residues at the surface of the cell membrane. Although we assume that the particles are internalised, this process is saturable, as indicated by the concentration results.

In view of the promising activity seen in the cell lines in vitro, we evaluated the particles in human tumour xenografts in vivo. Immunodeficient animals have the advantage that we can study the same human tumour cells that we use for the in vitro studies in an in vivo model. Tumours established from the drug-sensitive A2780 cell line show a marked growth inhibition when treated with the maximum tolerated dose of paclitaxel (15 mg/kg, i.v.  $\times 3$ ; Fig. 1). Paclitaxel has very limited bioavailability when given by the oral route. However, although not as effective as the standard treatment regimen, the orally delivered paclitaxel nanoparticles showed activity in this model. This result differs from that of an earlier study in chemically induced rat tumour model, where nanoparticulate formulation at half the dose was equivalent to the micellar one (Bhardwaj et al. 2009). The drug-resistant derivative of A2780 (2780 AD) has the MDR phenotype and is very resistant to the group of drugs involved (Plumb et al. 1990). Although this is an in vitro derived drug-resistant cell line, it provides a useful model to evaluate strategies to circumvent drug resistance mechanisms. We have shown that sensitivity to paclitaxel can be increased by treatment with an inhibitor of the drug efflux pump (Mistry et al. 2001). Tumours derived from 2780 AD are resistant to the maximum tolerated dose of paclitaxel (Fig. 2). However, these tumours showed a significant growth delay when mice were treated with the oral paclitaxel nanoparticles. Furthermore, in the mouse studies, the amount of the nanoparticle required to deliver the paclitaxel was nontoxic to the mice (Fig. 2).

The mechanism of the drug sensitisation is not clear. Possibly, the entrapment of the drug inside the nanoparticles shields it from the efflux transporters. In comparison, when the free drug is absorbed by the cells, it is more actively pumped out from the cell. The difference in this gradient gives credence to particle-trafficking mechanisms via structural engulfment rather than hypothesis of Fickian diffusion across the intracellular and paracellular routes. Also, since tubulin is one of the essential components of the structural architecture of cell, there is a higher chance of the bound paclitaxel to escape the efflux pump and contribute to tubulin depletion within the cell. Furthermore, due to the enhanced permeation and retention (EPR) effect of the tumour environment (Maeda 1992), the particles are expected to accumulate and produce a constantly available supply of sheathed paclitaxel to the cancer cells.

The relative inferior efficacy of the nanoparticles compared with the standard formulation of paclitaxel could be explained by a difference in the oral uptake of the drug. Rapidly multiplying cells generally exhibit higher sensitiv-



**Fig. 2** The effect of paclitaxel and paclitaxel nanoparticles on the growth of tumours of cell line 2780 AD when grown as xenografts in mice. Mice were treated once tumours reached a mean diameter of 0.5 cm. Groups of six mice were either untreated (●) given paclitaxel (dissolved in cremophor EL, ethanol, dextrose) intravenously (▼) empty nanoparticles orally (■) or paclitaxel-loaded nanoparticles orally (◆) on days 0, 2, and 4. Tumour volumes (A) and body weights (B) are expressed relative to that on the day of treatment (day 0), and results are the mean  $\pm$  SEM of six mice

ity to a cytotoxic drug compared to those multiplying slowly (Lopes et al. 1993). The absolute cell kill in that case is proportional to the drug concentration presented which shall be higher for the i.v. formulation compared to the oral formulation. Slowly multiplying cells, on the contrary, would require a sustained exposure of the drug, which will be provided by a formulation that can protect the drug from degradation.

## 5 Conclusion

The presented study provides an exciting strategy to improve the sensitivity of drug-resistant cancers especially of those for which resistance is mediated via over-expression of efflux transporters. The results in tumour model in nude mice demonstrate the possibility to deliver paclitaxel by oral route and the prospect of such formulations in increasing response to anticancer drug therapy.



**Acknowledgments** V. Bhardwaj is grateful for PhD studentship to the Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde.

## References

- Bhardwaj V, Ankola D, Gupta S, Schneider M, Lehr CM, Kumar M (2009) PLGA nanoparticles stabilized with cationic surfactant: safety studies and application in oral delivery of paclitaxel to treat chemical-induced breast cancer in rat. *Pharm Res* 26:2495–2503
- Cuvier C, Roblot-Treupel L, Millot JM, Lizard G, Chevillard S, Manfait M, Couvreur P, Poupon MF (1992) Doxorubicin-loaded nanospheres bypass tumor cell multidrug resistance. *Biochem Pharmacol* 44:509–517
- Dantzig AH, Shepard RL, Cao J, Law KL, Ehlhardt WJ, Baughman TM, Bumol TF, Starling JJ (1996) Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. *Cancer Res* 56:4171–4179
- de Verdière AC, Dubernet C, Némati F, Soma E, Appel M, Ferté J, Bernard S, Puisieux F, Couvreur P (1997) Reversion of multidrug resistance with polyalkylcyanoacrylate nanoparticles: towards a mechanism of action. *Br J Cancer* 76:198–205
- Dye D, Watkins J (1980) Suspected anaphylactic reaction to cremophor EL. *Br Med J* 280:1353
- Eldridge JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, Tice TR (1990) Controlled vaccine release in the gut-associated lymphoid tissues: I. Orally administered biodegradable microspheres target the Peyer's patches. *J Control Rel* 11:205–214
- Emilienne Soma C, Dubernet C, Bentolila D, Benita S, Couvreur P (2000) Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles. *Biomaterials* 21:1–7
- Florence AT (1997) The oral absorption of micro- and nanoparticles: neither exceptional nor unusual. *Pharm Res* 14:259–266
- Kabanov AV, Batrakova EV, Alakhov VY (2002) Pluronic® block copolymers for overcoming drug resistance in cancer. *Adv Drug Delivery Rev* 54:759–779
- Lopes NM, Adams EG, Pitts TW, Bhuyan BK (1993) Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell lines. *Cancer Chemother Pharmacol* 32:235–242
- Maeda H (1992) The tumor blood vessel as an ideal target for macromolecular anticancer agents. *J Control Rel* 19:315–324
- Mistry P, Stewart AJ, Dangerfield W, Okiji S, Liddle C, Bootle D, Plumb JA, Templeton D, Charlton P (2001) In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* 61:749–758
- Plumb JA, Milroy R, Kaye SB (1989) Effects of the pH dependence of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Res* 49:4435–4440
- Plumb JA, Milroy R, Kaye SB (1990) The activity of verapamil as a resistance modifier in vitro in drug resistant human tumour cell lines is not stereospecific. *Biochem Pharmacol* 39:787–792
- Romsicki Y, Sharom FJ (1999) The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporters. *Biochemistry* 38:6887–6896
- Roy SN, Horwitz SB (1985) A phosphoglycoprotein associated with taxol resistance in J774.2 cells. *Cancer Res* 45:3856–3863
- Sakuma S, Hayashi M, Akashi M (2001) Design of nanoparticles composed of graft copolymers for oral peptide delivery. *Adv Drug Delivery Rev* 47:21–37
- Soma CE, Dubernet C, Barratt G, Nemati F, Appel M, Benita S, Couvreur P (1999) Ability of doxorubicin-loaded nanoparticles to overcome multidrug resistance of tumor cells after their capture by macrophages. *Pharm Res* 16:1710–1716
- Thierry AR, Vige D, Coughlin SS, Belli JA, Dritschilo A, Rahman A (1993) Modulation of doxorubicin resistance in multidrug-resistant cells by liposomes. *FASEB J* 7:572–579
- Valle J, Armstrong A, Newman C, Alakhov V, Pietrzynski G, Brewer J, Campbell S, Corrie P, Rowinsky E, Ranson M (2010) A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronic, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction. *Investig New Drugs*. doi:10.1007/s10637-010-9399-1
- Warren L, Jardillier J-C, Malarska A, Akeli M-G (1992) Increased accumulation of drugs in multidrug-resistant cells induced by liposomes. *Cancer Res* 52:3241–3245
- Wong HL, Bendayan R, Rauth AM, Xue HY, Babakhanian K, Wu XY (2006) A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system. *J Pharmacol Exp Ther* 317:1372–1381
- Workman P (2010) Guidelines for the welfare and use of animals in cancer research. *Br J Cancer* 102:1555–1577